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PERSONAL &
CONFIDENTI

To: .Dr. C. K. Ellis

Date: July 24, 1988

From: .R. D. Kinser

Subject: .TSNA Priority Program Operational Plans: Revised after Second Quarter 1988 Planning Meeting

OBJECTIVE: To design a product by 1991 with MS TSNA (TSNA/mg TPM) delivery reduced 90% relative to the TPM corrected TSNA delivery of a 1987 full-flavored, blended cigarette.

STATUS AND BACKGROUND

Previous studies have indicated that mainstream (MS) TSNA arise from pyrosynthesis during smoking and transfer of filler (endogenous) TSNA into the smoke stream, and that the total delivery is also affected by some TSNA decomposition during the smoking process. Increased understanding of the formation of TSNA during curing has been obtained from two extensive curing studies, but current work aimed at reduction of TSNA transfer (distillation) is focused on selective removal of TSNA from cured filler. A continuous process whereby TSNA and alkaloids are extracted from filler and then removed from the extraction solvent by an ion exchange resin has been developed. The possibility of reducing endogenous TSNA by biochemical alteration of tobacco, resulting in lowered biogenesis of alkaloids, is being examined.

Research on the inhibition of TSNA pyrosynthesis has indicated that the amine precursors of NNN and NAT are the secondary amines nornicotine and anatabine. The amine precursor of NNK has not been identified, but our research indicates that nicotine is not the primary amine precursor of MS NNK. Disproportionately high levels of MS NNK from base webs and base webs extracted with organic solvents suggest the possibility of a "bound" form of an amine, such as "unextracted nicotine", may be the NNK precursor. Experiments to investigate this hypothesis have been initiated. Although addition of a primary amine to tobacco does not appear to be a viable means of reducing MS TSNA (NNN, NAT, and NAB are actually significantly increased), the small reduction in MS NNK following such addition will be examined further. Model studies have indicated that antioxidants accelerate TSNA thermal decomposition, that ascorbyl palmitate is more effective than propyl dihydroxyhydrocinnamate, and that NNN and NAT are more readily decomposed than NNK. TSNA pyrosynthesis does occur during the smoking of oriental (Ori) tobacco when nitrate and alkaloid levels are increased to approximate the levels of these probable precursors in burley tobacco. However, the smoke from an RL made from burley and oriental CELs applied to Bu base web delivers less MS TSNA than the smoke from an RL containing the same amount of Bu CEL and no Ori CEL. Studies to further evaluate the "oriental inhibitor" are planned. Cigarette circumference appears to have no effect on MS TSNA delivery on a gram filler consumed basis. Paper porosity and packing density will also be evaluated.

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STRATEGIES

These plans assume that Philip Morris chooses to not exert significant influence on tobacco cultivation, and therefore concentrate on tobacco treatment methods for decreasing TSNA delivery by distillation and methods which inhibit TSNA pyrosynthesis. Control of TSNA formation requires a greater understanding of those processes than currently possessed by us or described in the scientific literature, and the plans formulated to address the various strategies include defining the mechanisms of TSNA pyrosynthesis and of TSNA transfer into mainstream smoke. This basic research also includes a strategy designed to evaluate the possibility of TSNA reduction by biochemical alterations to the tobacco plant. The plans also include examination of various a priori methods for TSNA reduction. The target date represents our best prediction for a development model without flavor optimization meeting the 90% reduction (relative to a 1987 full-flavored, blended cigarette) goal using technologies and knowledge not available at this time. The priority assigned to each strategy, indicated by the number preceding the strategy, is based upon discussion with you and Dr. Sanders. With the exception of the strategy concerning oriental tobacco, this represents no significant deviation from the average of the priority assignments made by the scientists working in the program. Tactics will be designed for achievement of Strategy 9 as more information about various additives becomes available.

REDUCTION OF MS TSNA BY INHIBITING THE PYROSYNTHESIS OF TSNA

1. Reduce the levels of pyrosynthesized MS TSNA by removal of the amine precursor(s), or decreasing the reactivity to nitrosation of the amine precursor(s).

2. Reduce the levels of pyrosynthesized MS TSNA by incorporation into the cigarette design those aspects of oriental filler which result in an absence of significant TSNA pyrosynthesis from oriental tobacco.

3. Reduce the levels of pyrosynthesized MS TSNA by removing nitrosating agent(s) or precursor(s) of nitrosating agent(s), or blocking reaction pathways which form nitrosating agent(s) or which yield TSNA from the nitrosating agents.

REDUCTION OF MS TSNA BY REDUCING ENDOGENOUS TSNA IN FILLER

4. Reduce MS TSNA by selective removal of TSNA from filler.

5. Reduce MS TSNA by decreasing endogenous TSNA by biochemical alteration(s) to tobacco.

REDUCTION OF MS TSNA BY ENHANCING DECOMPOSITION OF TSNA

6. Evaluate the enhancement of TSNA decomposition during smoking as a method for reducing TSNA delivery.

REDUCTION OF MS TSNA BY ALTERING PHYSICAL/CHEMICAL PARAMETERS OF CIGARETTES

7. Reduce the levels of pyrosynthesized MS TSNA by alterations in cigarette construction parameters.

8. Reduce the levels of pyrosynthesized MS TSNA by manipulation of filler salt content.

9. Reduce the levels of pyrosynthesized MS TSNA by manipulation of additives typically used in cigarettes but missing from the reference cigarette.

TACTICS AND TIMETABLE

Outlined below are detailed plans for the remainder of 1988 and an overview of work planned for 1989 and 1990. Timeframes given are best estimates possible at this time and represent updates based on review of this plan at the end of the Second Quarter, 1988. No attempt has been made to allow time for possible analytical or instrumental problems; any schedule revisions needed due to these causes will be made on a quarterly basis. These plans do not include investigations of methods modifications which have been assigned lower priority and which will be evaluated as time permits. Also, research areas which appear promising at this time may be found non-productive and therefore be eliminated. Similarly, results of the earlier studies will surely suggest tactics and possibly even strategies which have not yet been considered; these plans will be updated as ideas develop. Included in this revision are requests received to date from other priority programs for TSNA analyses.

THIRD QUARTER 1988

Amine Precursor Strategy

Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by the following:	
Extract green tobacco with hexane and cure	begin at harvest
Complete study of base web and unextracted nicotine	Sept. 30
Addition of models of unextracted nicotine	
Nicotine release compound	Sept. 30
Investigate nicotine/water insoluble protein connection	Initiate Aug. 1
Evaluate effect of primary amine on MS NNK from BW	Sept. 6
Study other primary amines as competitors	as needed
Continue evaluation of other alkaloids and derivatives which may be precursors:	
Develop assay for PsON	Sept. 30
Evaluation of MS TSNA from SCFE tobacco with depleted secondary amines (minor alkaloids)	Sept. 30

Oriental Inhibitor Strategy	
Determining the effect of "local" cultivation on chemistry of oriental tobacco:	
Completion of "local" cultivation of oriental	Sept. 1
Evaluate effect of antioxidant incorporated in Bu/Ori RL	Sept. 15
Continuation of Tactic 2 from strategy 2 (if warranted):	
Tactic #2: Determine the reasons oriental does not yield significant levels of pyrosynthesized TSNA by evaluation of the following:	
CEL fractions in model experiments	Sept. 15
Nitrosating Agent Strategy	
Initiate implementation of nitrosation proposal	Aug. 22
Extraction of Endogenous TSNA Strategy	
Testing extraction/TSNA removal/addition process	Aug. 31
Subjectives on cigarettes from extracted fillers	Sept. 30
MS TSNA from cigarettes from extracted fillers	Sept. 30
Project ART support	
TSNA in water used as adsorber	Aug. 15
Support of Sepracor program	on-going
TSNA in ART products	on-going
TSNA in ART processing (traps, vessels, adsorbers)	on-going
Biochemical Alterations to Tobacco Strategy	
Characterization of PMT	
PMT stability	July 31
PMT enzyme kinetics	Aug. 31
Begin negotiations with Calgene	June 29
Optimize classical techniques and prepare enriched PMT fraction (100 μg; 1000-fold)	Sept. 30
Communicate PMT preparation methods to Calgene; initiate development of proposal for further work	Sept. 30
Monoclonal antibody preparations from Hazleton for screening at PM	July 1
Determine presence of PMT-MAb	Aug. 31
Obtain sufficient quantity of PMT-MAb	Oct. 31
TSNA Decomposition Strategy	
PrDHHC examined at higher temperature	July 15
Complete proposal for future research	July 31
Slow heating of antioxidant/TSNA mixtures	Aug. 15
Initiate implementation of homolytic cleavage proposal	Sept. 1
Cigarette Construction Parameter Strategy	
Evaluate effect of paper porosity and packing density	as time permits

Support to other Priority Programs

Develop simultaneous VNA and TSNA gc analysis	Aug. 5
Analyze new products as requested	on-going

FOURTH QUARTER 1988

Amine Precursor Strategy

Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by the following:	
Complete analyses of cured, extracted green tobacco	Dec. 31
Addition of microencapsulated nicotine as model for unextracted nicotine	Dec. 31
Continue nicotine/water insoluble protein studies	Dec. 31
Continue evaluation of other alkaloids and derivatives which may be precursors:	
Determine PsON in fillers	Nov. 15
Study polymeric secondary amines as precursors	Dec. 31
Replicate Hoffmann ^{14}C-nicotine addition: methods development	Dec. 31

Oriental Inhibitor Strategy

Determining the effect of "local" cultivation on chemistry of oriental tobacco:	
Analysis of samples from "locally" cultivated oriental	on-going
Evaluate MS TSNA delivery from an RL composed of oriental CEL and CEL from SCFE burley on Bu base web	Nov. 1
Initiate study of salt content and oriental "inhibitor"	Nov. 15
TSNA from CEL fractions in smoking experiments	Nov. 30

Nitrosating Agent Strategy

Continue studies outlined in nitrosation proposal	on-going

Extraction of Endogenous TSNA Strategy

Evaluate samples from water condensation from CO_2 from SCFE program	Dec. 30
Investigate photolysis of TSNA as means of removal from extracting solvents	Dec. 15
Perform necessary studies in support of Sepracor program	on-going

TSNA Decomposition Strategy

Conduct smoking experiments with optimized antioxidant levels	Dec. 31
Characterize decomposition pathway from kinetic/thermodynamic perspective	Initiate
Effect of altered burn temperature on decomposition	Dec. 31

Cigarette Construction Parameters Strategy

Construction parameters studies	on-going

1989

Addition of ^{14}C-nicotine to filler and effect on smoke TSNA
Effect of removal of alkaloid/amines by various processes on MS TSNA
Effect of trace metal content on MS TSNA delivery
Effect of pH of oriental
Initiate studies of role of NO in nitrosation
Completion of nitrosating agent studies
Evaluation of salt effects
Complete construction parameters studies

1990

Construction and evaluation of models based upon studies to date

RESOURCE ALLOCATIONS FOR 1988

How are the personnel assigned to this program allocated?

Amine Precursor Strategy:	Haut 65% Warfield 15% Kaiser 55% Kurth 55% Lambert 10% Kinser 15%
Oriental Inhibitor Strategy:	Morgan 5% Haut 15% Warfield 20% Kinser 10%
Nitrosating Agent Strategy:	Morgan 50% Kaiser 15% Kurth 15% Haut 10% Kinser 10%
Extraction of Endogenous TSNA Strategy:	Warfield 60% Tickle 25% Lambert 15% Kurth 20% Kinser 10%
Biochemical Alteration of Tobacco Strategy:	Nakatani 50% Crockett 100% Dunn 100% Malik 100% Mooz 70% Sykes 100% Yu 100%
Decomposition of TSNA Strategy:	Morgan 35% Tickle 35% Kinser 10%

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Cigarette Construction Parameters Strategy: Lambert 60%
Kaiser 15%
Kinser 5%
Morgan 10%

Adjustments to Filler Salt Content Strategy: None in 1988

Are there enough people allocated to this program?

Project 6908 Activities:

Two professionals assigned to this program in 6908 are currently working nearly full time on TSNA aspects of two other priority programs. An additional professional is on temporary assignment in Project Delta. While short term research plans have been adjusted accordingly, the long term effect of these alternate activities will likely be a delay in achievement of program objectives. Assignment of an Associate Scientist A/B to this program to assist in the support to other priority programs would significantly increase the probability of developing the reduced TSNA cigarette by 1991.

A new Research Technician III joined Project 6908 on June 20, 1988, to address a previously identified need.

Project 1904 Activities:

A biochemist with experience in immunology research is needed in addition to the personnel already assigned to this program. Applicants are currently being interviewed for this position, as well as for an Associate Scientist B to provide technical support for these studies.

Are the people allocated to this program the right people in terms of skills?

In terms of skills, the current staffing of this program appears adequate through 1989, with one exception. Four senior professionals with formal training and years of research experience in organic chemistry and analytical chemistry are assigned to this program in 6908. Three (including Ms. Hansen) junior professionals and one technician, all with significant on-the-job training in the appropriate analytical methods and all assigned to Project 6908, capably assist in the fundamental research programs and perform analyses requested by colleagues in other parts of R&D. One technician is currently in training, and should be independently performing some support functions by the end of the third quarter. Four senior professionals and two junior professionals with research experience in several biological disciplines are involved in the Project 1904 studies of biochemical alterations of tobacco. Additional expertise in biochemistry/immunology is needed in this area.

Are there special equipment and facilities and/or outside expertise required?

Other than the specialized analytical equipment already in our laboratories, the only other special equipment we foresee needing is available in the supercritical fluid extraction facility in the Physical

Research Division and the greenhouse facility in the Chemical Research Division. Outside consultants in the area of nitrosation chemistry and/or N-N bond cleavage may be needed; the decision is dependent upon the results of literature searches planned for 1988. A contract with Hazleton Biotechnologies Company has been obtained to facilitate the research on biochemical alterations to tobacco.

IMPACT ON OTHER AREAS BOTH WITHIN AND OUTSIDE R&D

Research described in this plan will require specific assistance from the Analytical Research Division, the Chemical Research Division, and the Physical Research Division. For 1988, requests to Analytical should require 0.25 to 0.5 man-months to complete. Extractions to be requested from the Physical Research Division's supercritical fluid facility during 1988 also should be completed within 0.5 man-months. Growth of tobacco plants needed for the biochemical alterations of tobacco research will require 0.6 man-year of support from the greenhouse staff. Also part of this study is a $32,000 contract of 6 - 9 months duration with Hazleton Biotechnologies Company. Replication of experiments describing the formation of NNK reported by scientists at the American Health Foundation will require an estimated six man-months of work by members of Chemical Research. Ultimately machine-made cigarettes will be required for evaluation, but that will probably not be until 1990. Assistance and training from individuals more skilled in cigarette design than anyone currently working in this area will be required when model construction and evaluation becomes the primary task of this group.

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